Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution for the system suitability test. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:
$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left(\frac{t_R}{w_h}\right)^2$$

where:
n=Efficiency, as number of theoretical plates for column;
t_R=Retention time of solute; and
w_h=Peak width at half-height.

(3) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:
t_{Rj}=Retention time of a solute eluting after i (t_{Rj} is larger than t_{Ri});
t_{Ri}=Retention time of any solute;
w_i=Width of peak at baseline measured by extrapolating the relatively straight sides to the baseline of any solute; and
w_j=Width of peak at baseline measured by extrapolating the relatively straight sides to the baseline of any solute eluting after i.

(4) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:
$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, except alternate injections of the working standard solution with injections of the sample solution.

[50 FR 48397, Nov. 25, 1985]

§ 436.357 Atomic absorption test for sodium carbonate content.

(a) *Equipment.* A suitable atomic absorbance spectrophotometer equipped with:

(1) A suitable sodium hollow-cathode discharge lamp;

(2) An oxidizing air-acetylene flame;

(3) A nebulizer-burner system;

(4) An optical dispersing device capable of isolating a resonance line of sodium from other wavelengths produced by the emission source; and

(5) A suitable radiation detector.

(b) *Ionization buffer.* Dissolve and dilute 19.07 grams of potassium chloride in distilled water to 1,000 milliliters.

(c) *Preparation of reference standard and sample solutions*—(1) *Reference*

standard solution. Accurately weigh approximately 140 milligrams of sodium chloride, which has been previously dried for 40 to 50 minutes at a temperature of 500 to 650 ° C. Dissolve and dilute with sufficient distilled water to obtain a stock solution containing 5.5 micrograms of sodium per milliliter. Mix 10 milliliters of the stock solution with 10 milliliters of ionization buffer and dilute the mixture with distilled water to obtain a solution containing 0.55 microgram of sodium per milliliter.

(2) *Sample solution*. Dilute the stock sample solution, prepared as directed in the monograph for the drug being tested, with distilled water to obtain a solution containing 5.5 micrograms of sodium per milliliter (estimated). Mix 10 milliliters of this solution with 10 milliliters of ionization buffer and dilute the mixture with distilled water to obtain a solution containing 0.55 microgram of sodium per milliliter (estimated).

(3) *Procedure*. Determine the atomic absorbance of the reference standard and sample solutions at a wavelength of 589 nanometers, using the atomic absorbance spectrophotometer and a reagent blank prepared by diluting 10 milliliters of ionization buffer to 100 milliliters with distilled water.

(d) *Calculations*. Calculate the percent sodium carbonate (S) as follows:

$$\text{Percent sodium carbonate} = \frac{A_u \times P_s \times 2.304}{A_s \times C_u \times 10}$$

where:

A_u=Absorbance of sodium in the sample solution;

A_s=Absorbance of sodium in the reference standard solution;

P_s=Sodium concentration in the reference standard solution in micrograms per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution.

[50 FR 48398, Nov. 25, 1985, as amended at 54 FR 20785, May 15, 1989]

§ 436.358 High-performance liquid chromatographic assay for pyridine.

(a) *Equipment*. A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 15 to 25 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure*. Perform the assay and calculate the pyridine content using the temperature, instrumental conditions, flow rate, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 25 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed for the system suitability test by five replicate injections of the same volume (between 10 and 20 microliters) of the system suitability test solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test*. Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor*. Calculate the tailing factor for the pyridine peak (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows: